Increased Homocysteine in a Patient Diagnosed with Marfan Syndrome

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CASE

A 53-year-old Caucasian woman was diagnosed in late childhood with Marfan syndrome according to characteristic skeletal features and bilateral lens dislocation. In addition, she has a history of nonischemic cardiomyopathy with severe left ventricular failure and atrial fibrillation, diabetes mellitus type 2, hyperlipidemia, progressive dementia, numbness in the lower extremities, and hypothyroidism following thyroidectomy for thyroid cancer. Additional findings revealed in a physical examination included an upper-to-lower segment ratio of 0.88, an arm span-to–height ratio of 1.02 (an upper-to-lower–segment ratio <0.85 and arm-span-to–height ratio >1.05 are 2 of the diagnostic criteria for Marfan syndrome), an elongated face, a high arched palate, and crowded dentition. She recently underwent further laboratory testing after a cardiologist did not find 2 characteristic features of Marfan syndrome, namely an enlarged aortic root and mitral valve prolapse. Her total plasma homocysteine and methionine concentrations were increased at 198 μmol/L (reference interval, 5–15 μmol/L) and 370 μmol/L (reference interval, 10–50 μmol/L), respectively. The patient’s plasma homocystine concentration was 48 μmol/L (reference interval, <2 μmol/L), and her urine homocystine concentration was also markedly increased. These biochemical abnormalities are not characteristic of Marfan syndrome. Her diagnosis was reconsidered in light of these new data.

DISCUSSION

In this case, a long-standing clinical diagnosis was inconsistent with recently obtained clinical and biochemical data. The biochemical data were virtually diagnostic of homocystinuria [OMIM3 (Online Mendelian Inheritance in Man) 236200], a term historically used to describe inborn errors of metabolism characterized by excessive excretion of homocystine in urine and high concentrations of total plasma homocysteine. Homocystinuria is most commonly caused by autosomal recessive inheritance of a deficiency in cystathionine β-synthase (CBS) activity (1). The worldwide incidence has been estimated to be between 1 in 200 000 and 1 in 335 000 population (2), with the highest incidence (1 in 1800) reported in Qatar (3). The diagnosis of homocystinuria in this patient was confirmed by sequencing of the CBS (cystathionine-beta-synthase) gene, which revealed 2 heterozygous missense mutations in exon 12 (c.1111 GÆA and c.1135 CÆT). These mutations cause the amino acid changes V371M and R379W, respectively, in the mature polypeptide.

Homocystinuria may occur in other pathologic conditions. These causes include (a) nutritional vitamin B12 deficiency, (b) acquired and inherited intrinsic factor deficiency, (c) selective intestinal malabsorption of vitamin B12, (d) transcobalamin II deficiency, (e) mutations in genes responsible for defects in intracellular methylcobalamin synthesis [MTRR (5-methyltetrahydrofolate-homocysteine methyltransferase reductase), MTR (5-methyltetrahydrofolate-homocysteine methyltransferase); also referred to as complementation groups cbIE and cbIG, respectively], and (f) N(5,10)-meth-

QUESTIONS TO CONSIDER

1. What are the molecular defects responsible for Marfan syndrome?
2. What pathologic conditions are associated with lens dislocation?
3. What conditions are associated with increased homocysteine in blood and urine?
yaleneterahydrofolate reductase deficiency. There may be coexisting methylmalonic aciduria in patients with combined intracellular methylcobalamin and adenosylcobalamin defects. These conditions include genetic defects in the genes MMACHC [methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria]; MMADHC [methylmalonic aciduria (cobalamin deficiency) cblD type, with homocystinuria]; and LMBRD1 (LMBR1 domain containing 1).

The symmetrical disulfide of homocysteine (reduced or thiol form) is termed “homocystine” (oxidized); both names indicate that each carbon chain of these compounds contains 1 methylene (CH₂) group more than those of cysteine and cystine, respectively (4). The upper reference limit of the total plasma homocysteine concentration in humans is 15 μmol/L, although there is some variation due to genetic factors, age, sex, menopausal status, and other physiological and lifestyle variables. Approximately 30% of the total homocysteine is in the free form, with the rest bound through disulfide bonds to cysteine residues in proteins, mainly albumin (4). Only 1% to 2% of the homocysteine that is not bound to proteins occurs as the thiol; the remaining 98% is in the form of disulfides (homocysteine–cysteine and homocystine) (4). In homocystinuria, the thiol homocysteine fraction can increase to 10% to 25% as the total concentration approaches 150 – 400 μmol/L.

The natural history of untreated homocystinuria includes the development of thromboembolic disease, ectopia lentis, developmental delays, osteoporosis, and other skeletal complications (1). Ectopia lentis, a common manifestation of homocystinuria and Marfan syndrome, can be due to subluxation (lens zonules are still in place) or dislocation (no zonules in place). Fibrillin is the major protein in the zonules holding the lens in place.

Differential diagnoses of ectopia lentis also include trauma, syphilis, sulfite oxidase deficiency, and Weill–Marchesani syndrome. A good medical history and physical examination will rule out trauma, syphilis, and Weill–Marchesani syndrome (5). Careful assessment of the anterior segment of the eye may provide a clue to the cause of ectopia lentis. In Marfan syndrome, the zonules are stretched, and the dislocation occurs in a supratemporal direction, whereas in CBS deficiency the zonules are detached/scrolled into the anterior surface of the lens (5). Thus, the dislocation is in an inferonasal direction. In the absence of definitive trauma, homocysteine measurement is indicated for all patients with lens subluxation or dislocation.

MARFANOID FEATURES IN HOMOCYSTINURIA
In addition to ectopia lentis, homocystinuria and Marfan syndrome share other common clinical findings, such as long-bone overgrowth, a high arched palate, a crowded dentition, and scoliosis. They differ substantially, however, with respect to other clinical manifestations, such as arterial and venous thrombosis (present in homocystinuria), developmental delay and cognitive dysfunction (homocystinuria), and aortic dilation (present in Marfan syndrome) (6).

Marfan syndrome is caused by mutations in the FBN1 (fibrillin 1) gene, whereas homocystinuria is caused in most cases by mutations in the CBS gene. Fibrillins belong to the extracellular matrix proteins, which include 3 fibrillin isoforms (fibrillin-1, fibrillin-2, and fibrillin-3) and the latent transforming growth factor β–binding proteins. Fibrillins contain 6 – 8 intradomain disulfide bonds, a calcium-binding epidermal growth factor–like (cbEGF) domain, and a transforming growth factor β binding protein–like (TB/8-Cys) domain. These domains are essential to the structural integrity and functional properties of fibrillins. The most common mutations reported to cause Marfan syndrome result in the deletion or generation of cysteines in these domains, leading to disrupted disulfide formation. Similarities between Marfan syndrome and homocystinuria may also involve cysteine modification. The formation of cysteine–homocysteine disulfides has been suggested to disrupt the intramolecular disulfide bonds in fibrillin-1 (7). Subsequently, the modified molecule folds improperly, becomes more susceptible to proteolysis, and loses the ability to bind calcium, thereby producing altered secondary structures. Homocysteinylation may also alter folding of tropoelastin, which is important for the maintenance of elastic fibers in skin, lung, and the aorta, and thereby interfere with the structure and function of these tissues.

HOMOCYSTEINE MEASUREMENTS
Methods for measuring total homocysteine include HPLC with fluorometric detection, gas chromatography–mass spectrometry, enzymatic methods, and immunoassay. These assays require the use of agents to reduce the disulfide bond and liberate the thiol homocysteine. Homocysteine in plasma or urine may be measured without prior disulfide reduction via classic amino acid analysis (ion-exchange chromatography with postcolumn detection of ninhydrin conjugates) or by liquid chromatography–tandem mass spectrometry.

NEWBORN SCREENING AND HOMOCYSTINURIA
Measurement of the methionine concentration in dried blood spots is commonly used for screening the newborn population for homocystinuria. In a recent study in Qatar (3), newborn screening of blood spots for total homocysteine via liquid chromatography–tandem mass spectrometry (without derivatization) was reported to be more sensitive than methionine measurement for identifying babies with homocystinuria. Studies have reported cases of classic and vita-
min $B_6$ (pyridoxine)-responsive homocystinuria with methionine concentrations that do not exceed those found in unaffected children. These vitamin $B_6$-responsive patients are, therefore, not detected in current newborn-screening protocols that rely on methionine concentration \(^{(8)}\). The arguments against using methionine measurement as a screening tool include high false-negative rates due to the timing of sampling (babies sent home early will likely be missed) and low methionine concentrations in breast milk compared with infant formulas. Methionine can also be increased in methionine adenosyltransferase I/III deficiency, $S$-adenosylmethionine hydrolase deficiency, glycine $N$-methyltransferase deficiency, generalized liver disease, and infants fed a formula rich in methionine.

The measurement of total homocysteine in blood spots has raised issues concerning its stability, but homocysteine in dried blood is reportedly stable for 24 h at room temperature, with small reductions of about 9% occurring after 28 days of storage \(^{(3)}\). This degree of instability may be acceptable for screening purposes and may therefore replace methionine in the near future for newborn-screening programs.

**GENETICS**

Currently, 153 mutations in the $CBS$ gene have been reported \(^{(9)}\). The 3 most common protein variants are I278T, T191M, and G307S. $CBS$ mutations have regional and ethnic distributions, as well as multiple functional consequences. The activity of the G307S mutation is not sensitive to vitamin $B_6$, whereas patients with the I278T mutation respond to vitamin $B_6$ administration. In one study, the T353M protein product was found exclusively in African Americans and was associated with the vitamin $B_6$-nonresponsive phenotype \(^{(8)}\).

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**Fig. 1. The homocysteine–methionine cycle.**

THF, tetrahydrofolate; DMG, dimethylglycine; SAM, $S$-adenosylmethionine (methyl donor); Vit$B_{12}$, vitamin $B_{12}$; MS, methionine synthase; BHMT, betaine–homocysteine $S$-methyltransferase; SAH, $S$-adenosylhomocysteine.
Clinical Case Study

POINTS TO REMEMBER

- Marfan syndrome is caused by alterations to fibrillin-1, which disrupt its intramolecular and intermolecular interactions. Fibrillin-1 is an important component of the extracellular matrix in many tissues. Abnormal fibrillin-1 contributes to the myriad of features in Marfan syndrome, including tall stature, long limbs and digits, dilation of the aorta, cardiac valve malfunction, and lens dislocation.

- The differential diagnosis of lens dislocation includes trauma, syphilis, Marfan syndrome, Weill–Marchesani syndrome, sulfite oxidase deficiency, and homocystinuria. Lens dislocation in homocystinuria may be related to modification of cysteine residues in fibrillin-1 by disulfide-linked homocysteine.

- Increased homocysteine concentrations in the blood and urine may be caused by nutritional cobalamin deficiency, malabsorption of cobalamin, inherited defects in cobalamin and folate metabolism, and cystathionine β-synthase deficiency. Cystathionine β-synthase deficiency is the most common cause of homocystinuria.

- In some cases of homocystinuria, residual cystathionine β-synthase activity may be maximized with pharmacologic doses of pyridoxine (vitamin B₆). In other cases, biochemical improvement may be achieved by betaine administration to stimulate an alternative pathway for homocysteine disposal.

- All patients with unexplained ectopia lentis should be screened for homocystinuria.

- False-negative rates in newborn-screening programs for homocystinuria may be improved by measurement of blood spot homocysteine rather than the current approach, which exclusively uses measurement of the methionine concentration in blood spots.

The 2 amino acid alterations (V371M and R379W) found in our patient are very rare, and both are associated with a response to vitamin B₆. The V371M enzyme mutation was previously described in a patient with Dutch and Australian ancestry, and the R379W alteration has been described in 1 patient from central Europe (10). The patient in this report is of mixed Caucasian and American Indian descent, and her blood homocysteine concentration decreased rapidly after treatment with vitamin B₆ (500 mg/day) and folic acid (5 mg/day).

TREATMENT

About half of all CBS-deficient patients respond to pyridoxine therapy. Dietary restrictions of methio-

nine, folic acid, cysteine supplementation, and betaine (which promotes an alternative homocysteine remethylation pathway; see Figure 1) have also been used, especially in patients unresponsive to vitamin B₆. If treatment is started early, complications and Marfanoid features can be ameliorated or even prevented. This patient was initially treated with pyri-
doxine, which reduced homocysteine concentrations drastically to 2.6 μmol/L and normalized the methionine concentration, but the dose had to be decreased owing to progressive preexisting paresthesia, which probably was a late complication of dia-
betes. She was switched to low doses of vitamin B₆ and betaine, but noncompliance with betaine necessi-
tated a return to the original dose of vitamin B₆ without further complications.

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Commentary

Gerard Berry* and Harvey Levy

The confusion between the diagnosis of Marfan syndrome and that of homocystinuria has been known since the classic report of Schimke et al. from Johns Hopkins in 1965 (1,2). Before the Johns Hopkins report, it was widely assumed that someone with ectopia lentis and long extremities almost certainly had Marfan syndrome. The Johns Hopkins report drastically changed this assumption. Having become aware of the similarities between Marfan syndrome and this recently discovered inborn error of metabolism, homocystinuria, the Johns Hopkins group screened urine samples from patients with ectopia lentis and/or other features of Marfan syndrome and found that 38 of the patients (from 20 families) had homocystinuria, not Marfan syndrome. The clinical case study described in this issue is an excellent illustration of the need to differentiate these 2 genetic disorders.

Because the Marfanoid habitus may be present in patients with Marfan syndrome and those with cystathionine β-synthase deficiency, diagnostic mistakes continue in the medical community. Keeping in mind several key facts will minimize this confusion: (a) Atherosclerotic cardiovascular disease and venous/arterial thrombi and emboli are key elements in homocystinuria (3) but are absent as primary features of Marfan syndrome; (b) cognitive impairment may occur in patients with homocystinuria (3) but is not a feature of Marfan syndrome; and (c) aortic dilation is a cardinal feature of Marfan syndrome but is absent in children and young adults with homocystinuria.

The establishment of the correct diagnosis of homocystinuria early in life is of paramount importance because a protein-restricted diet, vitamin B6, and/or betaine may eliminate the connective tissue and vascular complications, as well as death from a thrombosis or embolism. Some patients with homocystinuria may not manifest the classic phenotype (4). Thus, all patients with evidence of vascular disease at a young age should undergo testing to determine the concentration of total homocysteine in serum.

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References


Commentary

Michael J. Bennett*

Marfan syndrome and classic homocystinuria represent the top 2 in the list of differential diagnoses for a patient presenting with external features of tall stature, disproportionately long limbs, arachnodactyly, scoliosis, and, after ophthalmologic investigation, lens dislocation. This case report is of an individual who carried the diagnosis of Marfan syndrome for a period of 30–40 years before the eventual recognition that she really was affected with homocystinuria due to cystathionine β-synthase deficiency.

Although we tend to regard the measurement of homocysteine as one of the more recent additions to
clinical laboratory repertoires, methods for the measurement of free homocystine in the urine have been available for many years. It is not clear whether this particular patient was ever investigated for homocystinuria as a child. It may well be that inadequately sensitive colorimetric spot assays were available for investigation at the time and that homocystinuria was erroneously ruled out, leaving Marfan syndrome as a diagnosis of exclusion.

The case is an excellent example of how we should not label any patient for life with a genetically unproved condition, because medical knowledge expands and therapeutic modalities for the correct diagnosis frequently improve. Although these 2 conditions have a number of features in common, they differ appreciably in the cause of death. Marfan syndrome frequently causes aortic dissection, whereas homocystinuria leads to arterial or venous thrombosis. Appropriate and different medical monitoring is required.

The treatment options for the 2 conditions are also very different. Treatment for homocystinuria, including vitamin B<sub>6</sub> therapy, is very effective in some patients and has been around for many decades. Additional therapeutic intervention may include betaine or dietary restriction of methionine. This particular patient appears to be biochemically responsive to vitamin B<sub>6</sub>, and earlier treatment might have alleviated some of the relentless disease progression. Therapy for Marfan syndrome is still experimental. Angiotensin antagonists such as losartan demonstrate promise in reducing aortic stress.

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